

USING THE CRYOMED FREEZER FOR CRYOPRESERVATION OF SAMPLES

This procedure allows documentation when a sample is cryopreserved. The Cryomed can be used for both cryovials and bags. For this procedure one must follow all biohazard precautions. **STERILE**

TECHNIQUE SHALL BE USED IN THIS PROCEDURE

1. The Cryomed 1010 is composed of 3 separate units. They are the computer control, the flatbed printer, and the cryopreservation chamber.
2. The computer control unit has several "stock" cryopreservation programs but custom programs can be programmed into the device.
3. The computer control can be turned on by a rocker switch located in the left top rear of the machine. The flatbed printer can be turned on by a red rocker switch on the front top right of the device. There are no switches to be turned on or off on the cryopreservation chamber. It is directly controlled by the computer control.
4. Prior to using the Cryomed it is required to check there is enough liquid nitrogen in the tanks in the utility hallway. The Cryomed will use about $\frac{1}{4}$ of a tank per cryopreservation procedure.
5. Prior to using the Cryomed there are several things that need to be checked on the flatbed printer. Remove the white cap on the brown ink cartridge. Simply pull the cartridge up from the black holder and pull the white cap off. Place the brown cartridge back into the black holder. It simple slips into it. Next check the other cartridges and holders for binding. They sometimes bump into one another. They are not used for the cryopreservation process since we only use one channel of the 4-channel printer.
6. Determine the correct probe that is needed for the procedure being used. Bags require a bag probe (flat brown), 5 ml vials require a long probe (silver tube), and 1 ml vials require a short probe (silver tube). Check the probe connection in the cryopreservation chamber. Check to see that it is secure.
7. The computer and flatbed printer are now ready to be turned on.
8. When both are on the printer can be checked for calibration. To calibrate press the "cham" button on the computer control unit. The brown cartridge on the printer should move all the way to the left on the printer paper. It should end at the -180 line on the chart paper. Then press the "samp" button on the computer control unit. The brown cartridge should move to the 0 line on the chart paper. If the red cartridge does not line up on either the -180 or 0 line then it can be adjusted using the grey knob on the brown set of pen controls (bottom right on the printer). These usually do not need to be adjusted unless the device was bumped or disturbed in some way.
9. When the flatbed printer's calibration has been checked then it can be momentarily turned off until the rest of the start-up process is done. If the printer is left on the paper roller will continue to move paper onto the bed of the printer and it will be wasted.
10. At this time the program can be loaded into the computer control. There are several "stock" programs. They are 7.1, 6.1, (bag programs) and 2.1 (vial program). Determine which program will be used for your cryopreservation procedure. For bag programs 6.1 is used for bags

containing 30 ml or less total volume. For bags containing 31 ml to 72 ml program 7.1 is to be used. Vials containing 5 ml need to use program 2.1 with a long probe. This program can also be used for 1 ml vials but a short probe needs to be used.

11. To enter the program the following sequence needs to be entered into the computer control unit. First make sure the unit is on. Next push the "prog" button (top, left). Then push buttons "1", "2", and "3" in that order. You will then push the desired program number into the computer controller (2.1 or 6.1 or 7.1), when entered the desired program number will appear on the digital display. Please note there is no "." Between the numbers for programming, only push "21" or "61" or "71" to obtain the desired program. Next push the "ent" button (extreme bottom right). The program is now completely set in the computer controller.
12. The bag or vial can be placed into the cryopreservation chamber. See "placement of probe" procedure to accurately place the probe on your sample to be cryopreserved.
13. When the probe is correctly placed the door to the cryopreservation chamber can be closed. Please use care when closing the door, do not hit the rubber gasket with the door latch.
14. Next turn on the flatbed printer.
15. Push the "run" button on the computer controller. This will initiate the freeze program. Several other buttons will need to be pushed once the freezer has started. The next button to push is the "scan" button (located on the lower middle section of the computer controller). Next push the "prog/act" and "cham/samp" buttons located near the top center of the computer controller.
16. Label the printer recording paper with all the appropriate sample information.
17. The computer controller is now running on the first section of the freeze program. This part of the program will need to be manually advanced to the next section of the program. When the sample temperature reaches 4 degrees C the program must be advanced. This is done by pushing the "run" button on the computer controller again. The digital display should show that it is on the second section of the selected program.
18. From now on the computer controller will automatically advance through the programs sections. When the program is complete an alarm will sound.
19. The alarm can be silenced by pushing the "alarm" button on the bottom left of the computer controller or simply by shutting the computer controller off entirely. It is best to shut the computer controller off since this will shut off the liquid nitrogen valve to the cryopreservation chamber.
20. When the computer controller has been shut off then the cryopreservation chamber door can be opened. Use caution since the inner area of the chamber is very cold. Wear protective gloves. When removing the contents of the cryopreservation chamber make sure the temperature probe has not become frozen to the sample. Carefully but quickly remove the probe away from the sample.
21. The contents from the cryopreservation chamber can now be placed in the vapor phase of a liquid nitrogen freezer for permanent storage.
22. Advance the recorded run sheet on the flatbed printer and remove it from the printer.
23. Turn off the printer. Replace the white cap onto the ink cartridge by following step 5 but in reverse.

24. Leave the cryopreservation chamber door open to allow the frost to dissipate. The door can be closed later on when there is no moisture in the chamber.
25. Check the liquid nitrogen tanks to determine if there is an adequate supply left in the tank for the liquid nitrogen freezer. If there is not enough liquid nitrogen then contact Airgas for a refill.

NEEDED MATERIALS FOR THIS PROCEDURE

- Cryomed controlled rate freezer
- Bag compression plates
- Bag or vial probes